THE PATHOGENICITY OF *NOSEMA APIS* TO INSECTS OTHER THAN HIVE BEES

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(*Received for publication* 31 *October*, 1913)

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I. INTRODUCTION

Nosema apis is now well known as the minute, microsporidian parasite that causes a malady of hive bees, popularly known as 'Isle of Wight' disease, though it should be noted that several diseases of bees are often being confused under this general name. The organism, *N. apis*, is transferred from bee to bee by means of resistant spores in the dejecta of the infected host, which spores are taken up by other bees, either with food or drink, or during the various processes consequent on the communal life of the bees.

The Nosema spores absorbed by the bee liberate amoeboid forms termed planonts, which enter the epithelial cells of the gut and

develop rapidly, particularly in the chyle stomach of the bee where they undergo rapid, asexual multiplication, forming meronts. The functional derangements resulting therefrom are quite sufficient to bring about the death of the host in many cases. Should the host react successfully on the parasites, spore formation ensues, and the Nosema leave the host as highly resistant spores that are well adapted for extra-corporeal life, and that serve to perpetuate the species should they be able to reach a new host.

Some experiments have been carried out with insects other than *Apis mellifica*, and tend to show that the pathogenicity of *Nosema apis* is far from restricted to the hive bee. Many of the experiments were suggested by observation of the habits of various insects present when examination of bees was in progress. Although the numbers of the insects used were not as large as we could have wished, the results are of interest and importance.

II. EXPERIMENTAL INFECTION OF VARIOUS INSECTS

Experimental infections of members of the Hymenoptera, Lepidoptera and Diptera have been made.

A. *HYMENOPTERA.*

The experimental insects used were humble bees, mason bees and wasps.

(i) HUMBLE BEES (*Bombus terrestris*, *B. lapidarius*, *B. hortorum*, *B. venustus*, *B. latreillelus*). It has been shown previously by Dr. Graham Smith and ourselves that a Nosema is a natural parasite of humble bees (*Bombus spp.*). It seems to be a different species of Nosema from that so destructive to hive bees, and chiefly parasitises the malpighian tubules of the humble bees.

But humble bees belonging to apparently clean stocks, when kept in captivity and provided with food contaminated with excrement of hive bees containing *Nosema apis* spores, both can, and do, become infected with the parasite, and die from the effects thereof. Both meronts and spores can be found in the walls of the gut, while the malpighian tubules, as is usual with infections of *Nosema apis*, remain uninfected. Humble bees used as controls and supplied with pure food showed no Nosema.

(ii) MASON BEES. The colonies of mason bees used were

brought from abroad and were lodged in a piece of old wall. Some of the bees were isolated to act as controls. The rest were kept in a wall, screened off to prevent exit, and were supplied with honey and pollen contaminated with spores of *Nosema apis* from dead bees. The abundant food supply proved attractive and the bees fed somewhat greedily. On the fourth day after the first supply of contaminated food, it was noted that fewer bees flew out, and a few dead ones were found. On dissection, the alimentary tract of these bees was shown to be parasitised with meronts of *N. apis*. The dwindling of the bees continued, and some were found unable to fly. *N. apis* in the form of meronts and young spores was demonstrable on examination. Ultimately the two colonies supplied with *N. apis* died out, and the brood also was found dead. A few larvae showed Nosema spores, but the brood had been too long dead when recovered for the determination of young stages of the parasite. When both colonies were extinct, the controls that had been supplied with pure honey were examined. Seven-eighths of them had survived, and when these were dissected no parasites were found. The controls that died were also dissected, but as their food canals were empty, and their fat bodies greatly reduced, their deaths were ascribed to voluntary starvation.

(iii) WASPS (*Vespa germanica*). Two years ago we reported that a colony of wasps had been exterminated by introducing bees dead of Nosema into their nest. Since then, the experiment has been repeated, with similar results, and examination of recently dead wasps has shown that multiplicative stages (meronts) of *Nosema apis* were present in numbers in the alimentary tracts of the wasps.

Again, wasps collect dead and dying bees and carry them away as food for their larvae. Several cases are known to us in which the wasp broods have died out entirely as the result of a liberal diet of bees dead of Nosema. As before, the parasite underwent development in the body of its host, and in the majority of the wasps, adults and larvae alike, the host was killed before the life-cycle of the parasite was completed by spore formation. The hypothesis that the newer a parasite is to its host, the greater is its virulence, thus receives further support.

B. *LEPIDOPTERA.*

Certain common Lepidoptera were used for experimental purposes, controls being kept in each case.

(i) CABBAGE WHITE BUTTERFLIES (*Pieris brassicae*). Both larvae and imagines were used for experiment, as both could possibly acquire *Nosema apis* naturally with their food.

(*a*) An adult, ♂, was fed on the day it emerged from the pupa on syrup made with castor sugar and contaminated with spores from the gut of a bee. The butterfly was fed again three days later, and died on the fifth day after the first feed. Nosema spores and young stages of the parasite were found in the gut. The control died on the sixth day, and no parasites occurred in it.

(*b*) An adult, ♀, was fed on the second day after escape from the pupa on sugar syrup contaminated with spores. It refused to feed on the first day. It lived four days after emergence. A few Nosema meronts were found in the walls of the gut after death. A control lived six days.

(*c*) Larvae. Cabbage plants near a badly diseased hive were found spattered with bee excrement. Dead larvae of cabbage white butterflies also were found. Several experiments were then made, of which the one cited was typical. Eight larvae were collected from clean cabbage plants. Four were fed on a cabbage leaf smeared with honey in which an infected bee's gut had been emulsified, the remaining four on ordinary clean cabbage leaves.

One experimental larva died after two days with Nosema infection in its gut. Two, also containing Nosema, died on the following day. The remaining one pupated on the third day, but the imago never emerged. Of the four control larvae, all pupated and produced imagines in due course.

(ii) PEACOCK BUTTERFLIES (*Vanessa io*). On two occasions when bees were under examination for *Nosema apis*, peacock butterflies came into the laboratory, and after the usual aimless flutterings, settled on the viscera of dead bees and proceeded to suck honey from the contents. Both were captured and were examined after death. A second butterfly was captured on each occasion to act as control.

(*a*) Adult, ♂. It lived two days after capture. When

dissected, no stages of *Nosema apis* were found in the gut walls, and only a very few Nosema spores—probably those ingested with the food—were present.

(*b*) Adult, ♀. This was a rather large female. Soon after feeding, oviposition began, and the insect died at its termination. No Nosema was found in it.

The extremely small number of experiments prevents any definite conclusion being reached as to the pathogenicity of the protozoon to the insect. All that can be said is that a negative result was obtained in the two cases investigated.

(iii) CINNABAR MOTHS (*Callimorpha jacobeae*). Larvae of the cinnabar moths were used for experiment as they were observed on several occasions feeding on groundsel contaminated with bee excrement. The subjects experimented on were obtained from a locality where bee-keeping was not practised, and where the larvae seemed remarkably healthy.

Fifteen young caterpillars of the cinnabar moth were placed on a plant of groundsel growing in a flowerpot and covered by a bell jar to prevent escapes. The groundsel was watered with an emulsion of dead bees containing Nosema spores, filtered through coarse muslin that readily allowed the Nosema spores to pass through, but retarded the passage of the chitinous portions of the bees. The groundsel foliage was watered daily. The larvae fed on the groundsel in a quite normal way. Control larvae were kept under similar conditions but their groundsel was watered with ordinary tap water. These control larvae never showed any form of *Nosema apis*. The results of the experiment were:—

Three caterpillars, fed on infected groundsel, died two days after the first infective feed. Young stages of *Nosema apis* were present in their mid guts. The hind gut was not affected. The parasites were in the multiplicative phase, meront formation being in progress.

Seven larvae died on the fourth day. Of these, one contained young spores of *Nosema apis*, two had a very heavy infection of young meronts in an actively dividing condition, and the remaining four showed a few young stages of the parasite. The condition of the guts of the larvae resembled that seen in bees, and the varying degrees of infection among the larvae are parallel to what

we have so often found when examining a series of bees from one colony.

One larva died on the fifth day after the first feed, and contained meronts of *Nosema apis*. On the same day one pupated, and ultimately a deformed male imago issued from it. This insect lived three hours only. It was dissected as soon as possible after death, but no stages of *Nosema apis* could be recognised with certainty within it.

The remaining larvae died during the night and were too decomposed when examined to permit the detection of the planonts or meronts of *Nosema apis*, had they been present. No spores were found.

The life cycle of *Nosema apis* as seen in the larvae of the cinnabar moth was the same as that found in the hive bee. The spores gave rise to planonts, which became meronts in the epithelial lining of the alimentary tract, and these, in turn, produced spores.

(iv) GOOSEBERRY MOTHS (*Abraxas grossulariata*). Observations on a garden in a district heavily infected with apian microsporidiosis showed that, in one case, the foliage of gooseberry bushes near infected hives was spattered with the excrement of the bees, and the dejecta on the leaves contained spores of *Nosema apis*. Beneath the bushes a few dead larvae of the gooseberry moth were found, and as the body of one of these contained Nosema spores, experiments with larvae obtained from an uninfected district were commenced. Twelve larvae were divided into two sets of six. Both were fed on gooseberry twigs, the twigs being moistened daily with equal quantities of water, and of water containing Nosema spores, respectively. The results may be summarised thus:—

(*a*) *Larvae supplied with food contaminated by Nosema spores.* On the fifth day after the first infective feed, one larva died. Nosema spores were found in its intestine, toegther with meronts. These spores were fed to bees and reproduced the disease.

On the seventh day two more larvae were found dead. Thirty-six hours had elapsed since they were last seen alive. Decay had been rapid, and it was impossible to identify young stages of *Nosema apis*, and no spores were found. Thus it is uncertain whether these two larvae became infected.

One larva died on the eighth day. It contained both spores and meronts of *Nosema apis*.

Two larvae pupated on the tenth day. The pupae were small compared with those from the control larvae. One pupa was dissected on the twelfth day, and a few meronts were found in the body. The second pupa did not produce an imago. Whether this was the effect of the action of the Nosema cannot be stated with certainty.

(*b*) *Larvae feeding on uncontaminated food.* Two of the larvae were dissected. Neither showed any trace of *Nosema apis*. The remaining four pupated. Two pupae were dissected and examined. *Nosema apis* was absent, nor was any other parasite found in them. Of the remaining two pupae, one produced a beautifully shaped and marked female, but the second did not develop.

Morphologically, the Nosema present in the larvae of the gooseberry moth differed in no wise from the parasite as seen in bees, and its identity was established by feeding bees with the spores obtained from the caterpillars and thereby reproducing the disease.

C. DIPTERA.

The Diptera used for experiment were blow flies, crane flies and sheep keds.

(i) BLOW FLIES (*Calliphora erythrocephala*). During the examinations of bees for *Nosema apis*, it was noticed that blow-flies settled on the viscera of the bees and fed upon them. The flies were also often seen sucking up the sweet excrement voided on the alighting board and sides of the hive; they subsequently showed Nosema infection.

A number of pupae of *Calliphora erythrocephala* were dug up in a garden on March 26th, 1912. The adult flies began to emerge on March 28th, but could not be induced to feed on that day. On March 29th, the flies sucked a piece of meat with some Nosema spores on it. The following day they were supplied with moistened sugar contaminated with Nosema spores. They refused again to feed, but sat on the sugar most of the time.

From April 1st to April 9th, the blow flies hatched out at the

rate of one or two a day, and were formed into experimental and control sets. Special cases are now cited:—

Blow Fly 1 emerged from the pupa. It was provided with contaminated sugar and sucked it readily. It died nine days after emergence, and on dissection showed some young stages and a very few spores of *Nosema apis* in its gut. Infection of the Malpighian tubules also occurred.

Blow Fly 2 was smaller than Fly 1, and emerged a day later than it. It also lived nine days, while its control lived ten days. At death it contained a number of Nosema spores.

Blow Flies 3, 4, 5 and 6 lived from 7 to 10 days after emergence. All were fed on infected candy. Blow Fly 3 became infected, showing a few spores at death. Blow Fly 4 showed no form of *Nosema apis*. Blow Flies 5 and 6 contained a very few spores of *N. apis*. Blow Fly 7 showed no *Nosema apis*, but contained what is probably a new species of Nosema. Blow Fly 8 contained many young stages of *Nosema apis*, together with a fair number of spores. Blow Flies 9 and 10 contained no parasites.

No control blow fly was found to harbour microsporidia.

From the above experiments it can be inferred that a certain number of Calliphora are attacked by *Nosema apis*, and the latter can prove fatal to them if they are ingested by the insect with its food.

(ii) Crane Flies (*Tipula oleracea*). Two crane flies were noticed sucking the viscera of bees prepared for microscopical examination. The crane flies were captured, but refused to feed in captivity and died two days after capture. When dissected, a few young stages of *Nosema apis* were found. Crane flies caught in the open and used as controls showed no trace of microsporidian infection.

(iii) Sheep Keds (*Melophagus ovinus*). All the insects previously mentioned were able to acquire spores of *Nosema apis* naturally by means of their food, though the number of cases occurring in the open may not be very considerable. During the course of this investigation, a number of healthy sheep keds, *Melophagus ovinus*, from a prize Southdown flock, were sent to us. Though it was not very probable that these insects could obtain Nosema spores in nature, it was considered that it might be of

interest to see what effect, if any, was produced on them by *Nosema apis*. In order to ensure the ingestion of Nosema spores by the keds, spores from the guts of bees were smeared on to a limited area of the forearm of one of the writers and six keds were fed on this small area. When wounds were made by the bites, additional small drops of infected excrement were placed on them, and through the layer of spore-containing material the keds had to force their proboscides to get blood. Some of the keds after sampling the sweet excrement slowly sucked it up before taking much blood. All six fed well, the process taking nearly an hour in every case. Six control keds were fed on a carefully disinfected area of the other arm of the experimenter. The next day the treated keds were alive but not active, as it was a very cold day. They were fed again in the same way, all feeding well, but three being particularly greedy. A third feed was given on the next day, when the three weaker feeders of the previous day also fed slightly. Examination of the excrement of these three showed that they contained a fungus, as reported by one of us in 1910.* The other three showed no fungus in their faeces. At noon on the fourth day after the first feed, the three keds containing fungus were dead. Microscopical investigation showed that they contained large quantities of fungus in their Malpighian tubules and young stages of *Nosema apis* in their mid guts. Soon after mid-day, the remaining keds were noticed to be much more feeble, and between 3 p.m. and 9 p.m. that day all three died. Examination of fresh preparations of them showed the presence of meronts and empty sporocysts of *Nosema apis* in the gut contents. Stained preparations were also made and were confirmatory of the fresh preparations; they also contained young spores. When the control keds were dissected, no form of *Nosema apis* was observed in any of them. From this experiment, it is shown that Hippoboscid flies, *Melophagus ovinus*, became infected when supplied with spores of *Nosema apis*, which underwent developmental changes in their bodies.

The Glossinae or tsetse flies resemble the Hippoboscidae in their

* Porter, A. (1910). The Structure and Life-history of *Crithidia melophagia* (Flu), an Endoparasite of the Sheep Ked, *Melophagus ovinus*. Quart. Journ. Microsc. Sci., LV, pp. 189-224. Two plates.

mode of reproduction and blood-sucking habit. As is well known, the Glossinae are the flies that transmit sleeping sickness or trypanosomiasis of men and animals. We would suggest that a search should be made in the various Glossinae by competent observers, who are well versed by practical experience in the structure and life history of Microsporidia, for parasites allied to the Nosema so destructive to bees, and pathogenic also to mason bees, wasps, and the various Lepidoptera and Diptera cited above. Should such a pathogenic Microsporidian be found as a hyper-parasite in Glossinae, it would be a forward step in the solving of the problem of sleeping sickness.

III. SUMMARY AND CONCLUSIONS

1. *Nosema apis* has been proved pathogenic to Hymenoptera other than bees. It can multiply in the food canals of humble bees, mason bees and wasps, and can bring about the deaths of the hosts.

2. Contamination of plants with infected excrement occurs in the neighbourhood of badly infected hives. Such contaminated food is pathogenic to the larvae of cabbage white butterflies, cinnabar moths and gooseberry moths, in which *Nosema apis* produces destruction of the tissue of the food canal in the same way as in bees. Both imagines and larvae of these insects became infected with microsporidiosis when supplied with food contaminated with Nosema spores.

3. *Calliphora erythrocephala.* The blow fly becomes infected naturally by ingesting Nosema spores contained in the sweet excrement of bees. This infection has been repeated experimentally. Crane flies may also become infected.

4. A member of the Hippoboscidae, *Melophagus ovinus,* has been infected successfully with *Nosema apis,* which is pathogenic to the sheep ked. It is suggested that research be made by competent observers among the Glossinae for Microsporidian parasites allied to the Nosema of bees, and, possibly, equally pathogenic to the tse-tse flies that may harbour them.

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